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The use of nanosecond electron beam for the eggs surface disinfection in industrial poultry

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Abstract. Development of the technology bases of egg disinfection using surface irradiation is executed by the nanosecond electron beam. Experiments on shell eggs irradiation using the frequency nanosecond URT-0.5 accelerator were made. Determination of absorbed dose distributionwas executed. In case of irradiation by the electron beam with absorbed dose level 5 kGy, it is enough for the full disinfection of the surface and pores of egg shell. The absorbed dose in the egg at the expense of bremsstrahlung will not exceed 0.08 Gy which can't have essential action on protein. Irradiation of shell eggs batch in plastic package of 100 pieces pledged in an incubator together with an inspection lot of not irradiated egg was made. The percent of eggs deductibility and chickens survival of pilot and control batches were identical, indicating that there was no significant radio biological effect from irradiation of egg internal structures. The received chickens were grown up within the 5 weeks. It is established that the chickens who are grown up from the irradiated egg have no essential differences in development.

1. Introduction

At present, virtually the only way to reduce the microbial contamination of foods is the heat treatment. However, thermal sterilization leads to irreversible changes in the properties of raw materials, which are not always acceptable. Applied chemical methods, such as salting, sugaring, etc. lead to the same result, and use a lot of preservatives. Therefore, the heat pasteurization is widely used to increase the shelf life of foods, followed by cooling to temperatures at which the multiplication of microorganisms is difficult.

An alternative is radiation sterilization, due to the universality of the harmful effects of ionizing radiation on any biological objects. In this way, the absorbed dose (AD) of radiation sterilization does not exceed 25 kGy. However, the irradiation of the food may be accompanied by a variety of chemical reactions which may transform the organoleptic properties of the products.

Thus, it is necessary to set the limits of AD for irradiation of various products. For example, for fresh eggs, a level of AD≤3 kGy is recommended, which is close to the AD level for inactivation of the bacteria of the Salmonella group [1]. Irradiated food is marked with a special sign "radura", so that the buyer could choose whether to use irradiated products or not. Unfortunately, the radiation phobia is of great importance for the consumer choice.

In our view, the following approaches look promising to solve the problems of microbiological contamination of eggs and the consumer sentiment.

Firstly, this can be accomplished by proper electron energy selection, to choose such an AD distribution profile within the product, that will destroy, upon irradiation, all kinds of microbes, including the pathogenic ones both on the shell surface and in its pores as well as in the air chamber up to the under-shell membranes. In that way, there will be practically no exposure of the protein itself to the accelerated electrons.

Secondly, the ozone will be produced under the irradiation, which will also contribute to the disinfection of the surface, especially by irradiation of the eggs sealed in plastic containers. It is possible to sterilize the eggs after packaging by the radiation itself as well as by the creation of ozone at a concentration levels lethal to microorganisms in the packaging – radiation-chemical sterilization [2]. At the same time, it is possible to select the AD distribution profile within the egg so that its protein is not irradiated by electrons at all. It is important that the presence of sealed plastic containers allows us to solve the problem of re-semination of eggs during storage.

Both ways have their merits. Moreover, in the real technological process, both ways can be combined in different proportions. The disadvantage of irradiation sterilization is its high cost and heightened risk for the working staff. This risk can be significantly reduced by optimizing the radiation source. At present, nanosecond electron accelerators for the technologies [3] which significantly reduce the costs of the radiation source itself, as well as the costs of the personnel radiation protection have been developed and produced.

A number of authors [4, 5] found that a substrate processing with 3 MeV electron beam at AD=6.7 kGy is sufficient to reduce the total microbial contamination and the number of Coliform bacteria to the standard values. The range of the radiobiological effect manifestation for Enterobacteria made 2.7-30.7 kGy, the survival decreased with AD increase; a complete elimination of microorganisms was noted at 30.7 kGy. The study of spore form survival, conducted using the example of Bacillius, revealed a striking effect during the treatment of an electron beam in the range of 1.3-10.4 kGy.

Furthermore, a stronger bactericidal effect of the nanosecond electron beam (NEB) is known [6]. That will allow reducing the AD magnitude of the electron beam 2–3 times, which will increase the efficiency of the method while leaving the energy consumption and material costs the same [7].

Unfortunately, it is impossible to completely avoid irradiation of the egg protein, bremsstrahlung is induced by absorption of the electrodes which makes a major contribution to the AD, created inside the egg. Now we study the ranges of dose sensitivity for three pathogenic strains of Salmonella genus bacteria in vitro. The sterilizing effect of NEB for a chicken egg surface was analyzed, the influence of NEB on the physicochemical properties of an egg was studied. The radiobiological effect was studied using the example of hatching eggs.

2. Experimental

The experiments were carried out by the accelerator URT-0.5 [8] (electron energy up to 500 keV, pulse width ~50 ns, pulse repetition rate up to 200 pps).On the feeding conveyor the plastic containers for 10 eggs are passed through a uniform pulsed electron beam irradiation by width. After the processing the containers with eggs were inverted and the cycle was repeated.

AD of NEB was tested by a film dosimeter and AD of bremsstrahlung radiation was measure by TLD-500 dosimeters. The TLD-500 were placed in the sections of boiled eggs (figure1). During the experiments, the accelerator was operating at a charging voltage of 30 kV. During the processing of table eggs an ADs were 5 and 25 kGy, during the processing of hatching eggs the AD was 40 kGy.

The representatives of Enterobacteriaceae family are facultative anaerobes which do not form spores. Enterobacteria include the representatives of the normal microflora among humans and animals, saprotrophs, as well as a number of relatively pathogenic and pathogenic microorganisms, including the pathogens of serious infectious diseases among humans and animals. The most characteristic nutritional transmission mechanism of pathogenic enterobacteria is implemented through food and water, which conditions the crucial importance of food antimicrobial treatment to save their sanitary quality.

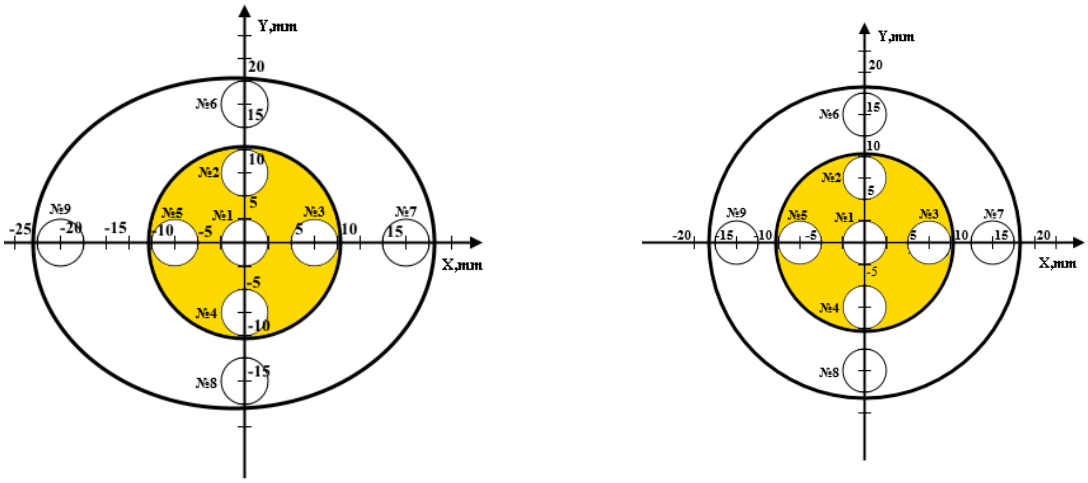

Figure 1. Location of TLD dosimeters (5 mm in diameter) at vertical (right) and the horizontal section of the chicken egg.

NEB disinfecting action efficiency on chicken eggs experiments were performed. Swabs were taken for bacteriological and bacterioscopic analysis from the test eggs of the control and experimental batches, then an experimental batch of table eggs in plastic packaging containers was subjected to an experimental irradiation by a NEB.

The control batch was in the same conditions as the experimental one, but it was not subjected to NEB action. In experimental batches, the AD was 5 kGy and 25 kGy. Immediately after the irradiation, swabs were taken from the egg surface and subjected to microbiological examination – sowing was carried out on nutrient media with the isolation of cultures and the identification of the obtained microorganisms. Eggs were stored for 25 days at the temperature of 0-20°C and the humidity of 85–88%. On the 12th and 25th days, the second and the third microbiological control of the experimental and control batches were carried out: the washing off from the surface of eggs, followed by the sowing on nutrient media, the isolation and identification of microorganisms.

The microbiological study of the hatchery eggs was carried out during the first day: prior to the impact by the beam of accelerated electrons and after it just before its laying in an incubator. The control batch was under the same conditions as the experimental one, but it was not exposed to NEB. The AD during egg processing of NEB was 40 kGy, which is 4 times higher than the maximum dose for food product radiation sterilization recommended by IAEA. The choice of a high AD is dictated by the need to evaluate the damaging effect of radiation on egg tissues.

In order to analyze the mass fraction of vitamin A in the yolk and vitamin B2 in the albumen, a high-performance liquid chromatography method was used.

The damaging effect of ionizing radiation is caused both by direct action on biomolecules and, indirectly, through the formation of oxygen active forms, which oxidize biomolecules. And this leads to the disruption of a cell vital activity [9]. The most radiosensitive cells are the cells of rapidly regenerating tissues, as well as the cells of embryos and fetuses.

In order to study the damaging radiation effect of a NEB on the hatchery egg tissues and a developing embryo, ovoscopy and macroscopic analysis of the quality of the egg were performed. Incubation was carried out according to the standard technology used in industrial poultry farming. The percentage of produced eggs and the quality of the young was assessed daily during the growing period before the slaughtering (37 days). They studied biochemical, immunological and hematological indices of broiler chicken blood to identify possible metabolic and physiological disturbances. A pathological anatomical study was conducted after the slaughtering of the experimental and control group chickens.

3. Results and discussion

3.1. Absorbed dose measurement results

The measurement results showed that during the irradiation by NEB with AD of 5 kGy level is sufficient for a full disinfection of a chicken egg surface, the AD within it will not exceed 8 cGy due to bremsstrahlung irradiation (table 1), which is not sufficient for the development of significant radiobiological effects in albumen and yolk.

Table 1. Measurement results of bremsstrahlung AD inside the egg (see figure 1).

	horizontal section								
Dosimeter number	1	2	3	4	5	6	7	8	9
AD, cGy/pulse	0.13	0.25	0.18	0.17	0.15	0.18	0.31	0.15	0.17
	vertical section								
Dosimeter number	1	2	3	4	5	6	7	8	9
AD, cGy/pulse	0.16	0.15	0.17	0.13	0.14	0.18	0.21	0,16	0.26

3.2. NEB disinfecting action efficiency on chicken eggs

The result showed that the processing of table 2 eggs in commercial plastic package with a NEB suppressed the growth of microflora on the surface of eggs completely with an AD of 5 kGy and more. If the storage conditions are observed, the surface of the eggs remains a sterile one throughout the whole regulated shelf life. The growth of St. aureus was noted in control samples.

Microbiological analysis of swabs from the hatchery egg surface of the experimental batch revealed a complete absence of microorganism growth.

Table 2. Microbiological evaluation of table egg processing efficiency by NEB method.

	Control, CFU/g			CFU*/g after NEB treatment		
AD, kGy	1st day	12th day	25th day	1st day	12th day	25th day
5	St.aureus, 400	St.aureus, 2900	St.aureus,16 000	No growth	No growth	No growth
25	St.aureus, 600	St.aureus, 660	St.aureus, 16 000	No growth	No growth	No growth

*The amount of Colony Forming Units (CFU), survived after NEB treatment with applied to the nutrient media of a standard suspension (1 billion of microbial cells per 1 ml).

3.3. The study of NEB effect on physical – chemical composition and structure of chicken eggs

It was found that the content of vitamin A in the egg yolk subjected to NEB was slightly higher than the content of a control batch of eggs (table 3). The content of vitamin B2 in the albumen of eating eggs was lower than the content of a control batch of eggs (table 3). The mass fraction of the shell

calcium in the experimental and control samples did not have statistically significant differences (table 4). The content of sodium in the shell was different, and was less in the control samples than in the experimental ones under the action of accelerated electrons (table 4). The content of phosphorus in a shell did not represent a reliable difference between all experimental and control samples.

The analysis of albumen substance content in eggs did not reveal statistically significant changes and differences between the samples of all experimental and control groups. During the study of amino acids, it was found that the content of lysine underwent greatest changes: the smallest amount was noted in control samples (0.88%±0.05%). The highest values were recorded in the samples subjected to the action of NEB (AD was 25 kGy) – (1.15±0.05)%. The changes in the percentage content of tryptophan and methionine were insignificant among all groups.

The internal structure of the hatchery eggs was examined by the methods of ovoscopy and visual evaluation. There is a tendency to increase the number of dots, rods and large pores on the shell, as well as the appearance of foreign inclusions in albumen and yolk during the 25th day as compared with similar indicators during the first day of the experiment.

Table 3. Vitamin content.

Absorbed dose,kGy	The content of vitamins in mg/kg			Control in mg/kg	
	5	25	40	Edible egg	Hatched egg
Vitamin A in yolk	2.83±0.10	2.59±0.11	3.41±0.17	2.79±0.10	4.12±0.17
Vitamin B_2 in albumen	3.55±0.42	2.24±0.12	–	7.06±0.31	–

Table 4. Content of elements in a shell.

Element	The content in mg/kg at AD		Control,mg/kg
	5 kGy	25 kGy	
Calcium	366112±18310		368192±18399
Sodium	1324	2064	908

All the revealed changes of shell, albumen, yolk and air cell qualities and properties corresponded to the natural changes in an egg structure during its long storage. They were relatively similar in the experimental and control groups. There were no statistically significant differences between the groups according to these characteristics.

3.4.Evaluation of NEB radiobiological impact on hatching egg embryo

A macroscopic analysis of the hatchery egg quality showed that the parameters of the albumen, yolk and eggshell from the control and test groups are very similar in value (table 5).

The ovoscopic assessment of eggs revealed insignificant differences in a shell structure: eggs with a large number of dots and rods on shell were larger in the experimental group than in the control group. The remaining parameters – the structure of albumen, yolk, air chamber, the presence of pathological inclusions did not have a statistically significant difference. After egg incubation, they calculated the percentage of withdrawal which was approximately identical (table 5).

A pathomorphological study of unborn chickens and the ones who died in the first hours showed that the reason for the chicken fading and death were the natural inaccuracies of incubation. There were no morphological signs of radiation damage and its consequences among embryos.

Table 5. Results of hatched egg quality microscopic analysis.

Parameter	Control	Test (40kGy)
Average diameter of protein, mm	79.2–80.8	
Height of egg protein, mm	7.6	
Protein index,%	1	
Yolk diameter, mm	43.5–44.5	
Yolk index, %	0.4	
Shell weight, g	7.5	7.4
Shell thickness, mm	0.33	0.32
Elastic deformation, μm	22	22.5
Number of dots and rods on shell, %	53	80
Hatch, %	63	64

Rates of body weight gain of chickens were in limits of standard values and were characterized by larger intensity in tested group in the first 14 days, and then these indicators in both groups were made.

Biochemical blood test noted an increase in the average value of the general protein and globulins content at control group chickens $p<0.05$ in comparison with similar indicators in skilled group.

Concentration of partateaminotransferase, the general creatine phosphokinase, glucose, uric acid, a lactate dehydrogenase, and also calcium and phosphorus of blood serum had no statistically significant distinctions between tests of tested and control groups.

4. Conclusions

On the basis of the stated above, it can be concluded that the use of a NEB for the disinfection of chicken eggs allows to eliminate the pathogenic microflora on the surface and in the pores of shell effectively without affecting the internal structures of an egg. The AD of 5 kGy is the sterilizing dose sufficient to kill the microorganisms on the surface of eggs in a plastic package. When you process eggs, at a AD exceeding the minimum sterilizing one in 8 times (40 kGy), no significant changes in the morphological physical-chemical properties ofalbumen, yolk, air cell and shell were detected.

The radiobiological effect is not expressed, since the hatchability and chicken health status indices obtained from the NEB treated eggs during the first hours of embryo development did not differ from the corresponding control group indicators.

Thus, the use of the NEB method for the disinfection of food and hatching eggs seems to be promising for industrial poultry farming, since it provides a pronounced bactericidal effect maintaining the food and incubatory qualities of an egg.The advantage of this method is the possibility of already packed product processing, which eliminates the repeated contamination of eggs with microorganisms. It is also possible to use NEB for the disinfection of containers, inventory and other objects of poultry farming.

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